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With this study, we explored the potential role of fungal endophytes in the antimicrobial activity of the medicinal plant goldenseal, *Hydrastis canadensis* L. (Ranunculaceae). A total of 23 fungal cultures were obtained from surface-sterilized samples of *H. canadensis* roots, leaves and seeds. Eleven secondary metabolites were isolated from these fungal endophytes, five of which had reported antimicrobial activity. *Hydrastis canadensis* plant material from the same harvest was analyzed for the presence of fungal metabolites using liquid chromatography coupled to high resolving power mass spectrometry. One fungal metabolite, the antimicrobial compound alternariol monomethyl ether, was detected both as a metabolite of the fungal endophyte *Alternaria* sp. isolated from *H. canadensis* seeds and as a component of an extract from the *H. canadensis* seed material. The concentration of this compound (991 mg/kg in dry seed material) was in a higher abundance than has previously been reported for metabolites of ecologically important fungal endophytes. The seed extracts themselves, however, possessed slight antimicrobial activity.

ANTIMICROBIAL COMPOUNDS FROM ENDOPHYTIC FUNGI OF GOLDENSEAL (*Hydrastis canadensis*)

by

Joseph M. Egan

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

This study focuses on endophytic fungi, which live asymptomatically within plant tissues, and the secondary metabolites that they biosynthesize. There has recently been a great deal of interest in endophytic fungi as a source for natural product drug discovery (Strobel, 2003; Suryanarayanan et al., 2009). It has been shown that endophytic fungi can have positive effects on their host plants, including improving drought tolerance, and producing protective compounds (Bush et al., 1997). Bioactive components from endophytic fungi have been shown to possess a number of different activities against human pathogens, and to possess novel cytotoxicity mechanisms (Strobel, 2003). It has also been shown that some fungal endophytes produce the same bioactive components found in botanicals they inhabit, which suggests that the influence of an endophyte may go beyond that of basic protection (El-Elimat et al., 2014; Kusari et al., 2013; Nisa et al., 2015; Stierle et al., 1993).

Because endophytic fungi are present in plant tissues, it has been suggested that compounds originating from endophytes may play a role in the biological activity of botanical extracts. There have been a few cases where this has been shown to be true for endophytic bacteria, which alter the *in vitro* anti-inflammatory activity of *Echinacea* extracts (Pugh et al., 2013; Todd et al., 2015). It is well known that fungal secondary metabolites can demonstrate potent biological effects, which supports the hypothesis that

fungal symbionts could also contribute to or alter the biological activity of botanical extracts. The goal of this study was to explore the potential role of fungal endophytes in the antimicrobial activity of the botanical medicine goldenseal, *Hydrastis canadensis* L. (Ranunculaceae).

It has previously been shown that *H. canadensis* has antimicrobial activity against a number of bacterial pathogens, and this activity has primarily been attributed to the alkaloid berberine (Hwang et al., 2003; Scazzocchio et al., 2001). However, several studies have demonstrated that the antimicrobial activity of goldenseal crude extracts is more pronounced than that of isolated berberine, suggesting that other compounds must also play a role (Ettefagh et al., 2011; Junio et al., 2011). With this study, we sought to isolate and identify fungal endophytes from *H. canadensis* plant material and determine whether their metabolites contribute to the antimicrobial activity of goldenseal botanical extracts.

CHAPTER II

RESULTS AND DISCUSSION

Endophyte Identities and Activities

Endophytic fungi, a total of 23 isolates, were cultured and identified from *Hydrastis canadensis.* The fungi were identified based on fragments of complete ITSrDNA; approximately 600-650 bp. Five isolates were identified as *Alternaria* sp., six as *Colletotrichum fioriniae*, three as *Diaporthe eres*, four as *Diaporthe* sp., two as *Sordariomycete* sp., one as *Magnaporthales* sp., one as *Phoma* sp., and one as *Pyrenocheta cava* (Table 1). A number of the fungal extracts possessed marked antimicrobial activity against *Staphylococcus aureus*, with the most pronounced activity observed for *Alternaria sp*. (Table 3).

Table 1. Identities of Endophytic Fungi. G numbers were assigned for in house identification, and OTU identification was performed via ITS rDNA sequencing

G#	OTU identification	Species identification of most homologous sequence from GenBank based on BLAST search*	Origin from Plant source	Reference
G09	*Diaporthe eres*	*Diaporthe eres (= Diaporthe cotonesteri; NR_119726)*	Leaf	(Udayanga et al., 2014)
G10	*Diaporthe* sp.	*Diaporthe terebinthifolii (NR_111862)*	Leaf	(Gomes et al., 2013)
G11	*Sordariomycete* sp. *(Xylariales)*	*Sordariomycete* sp. *NC1024 (JQ761762)*	Leaf	(U'Ren et al., 2012)
G12	*Colletotrichum fioriniae*	*Colletotrichum fioriniae (NR_117474, EF464594) (JN709486)*	Stem	(Damm et al., 2012; Shivas and Yu, 2009)
G13	*Alternaria* sp.	*Alternaria daucifolii (KC584193); Alternaria eichhorniae (NR_111832)*	Stem	(Woudenberg et al., 2013)
G14	*Diaporthe eres*	*Diaporthe eres (= Diaporthe cotonesteri; NR_119726)*	Stem	(Udayanga et al., 2014)
G15	*Diaporthe eres*	*Diaporthe eres (= Diaporthe cotonesteri; NR_119726)*	Stem	(Udayanga et al., 2014)
G16	*Diaporthe* sp.	*Diaporthe eucalyptorum (NR_120157)*	Root	(Crous et al., 2012)
G17	*Diaporthe* sp.	*Diaporthe eucalyptorum (NR_120157)*	Root	(Crous et al., 2012)
G22	*Phoma* sp.	*Phoma bellidis (GU237904)*	Leaf	(Aveskamp et al., 2010)
G23	*Magnaporthales* sp.	*Mycoleptodiscus terrestris (JN711860)*	Root	(Koo et al., 2012)
G28	*Alternaria* sp.	*Alternaria daucifolii (KC584193); Alternaria eichhorniae (NR_111832)*	Seed	(Woudenberg et al., 2013)
G29	*Diaporthe* sp.	*Diaporthe terebinthifolii (NR_111862)*	Seed	(Gomes et al., 2013)
G30	*Colletotrichum fioriniae*	*Colletotrichum fioriniae (NR_117474, EF464594) (JN709486)*	Seed	(Damm et al., 2012; Shivas and Yu, 2009)
G31	*Alternaria* sp.	*Alternaria daucifolii (KC584193); Alternaria eichhorniae (NR_111832)*	Seed	(Woudenberg et al., 2013)
G33	*Alternaria* sp.	*Alternaria daucifolii (KC584193); Alternaria eichhorniae (NR_111832)*	Seed	(Woudenberg et al., 2013)
G34	*Colletotrichum fioriniae*	*Colletotrichum fioriniae (NR_117474, EF464594) (JN709486)*	Seed	(Damm et al., 2012; Shivas and Yu, 2009)
G35	*Colletotrichum fioriniae*	*Colletotrichum fioriniae (NR_117474, EF464594) (JN709486)*	Seed	(Damm et al., 2012; Shivas and Yu, 2009)
G36	*Alternaria* sp.	*Alternaria daucifolii (KC584193); Alternaria eichhorniae (NR_111832)*	Seed	(Woudenberg et al., 2013)
G38	*Colletotrichum fioriniae*	*Colletotrichum fioriniae (NR_117474, EF464594) (JN709486)*	Seed	(Damm et al., 2012; Shivas and Yu, 2009)
G39	*Colletotrichum fioriniae*	*Colletotrichum fioriniae (NR_117474, EF464594) (JN709486)*	Seed	(Damm et al., 2012; Shivas and Yu, 2009)
G41	*Pyrenochaeta* sp.	*Pyrenochaeta* sp. *(EU885415)*	Leaf	(Ferrer et al., 2009)
G42	*Sordariomycete* sp. *(Xylariales)*	*Sordariomycete* sp. *NC1024 (JQ761762)*	Leaf	(U'Ren et al., 2012)

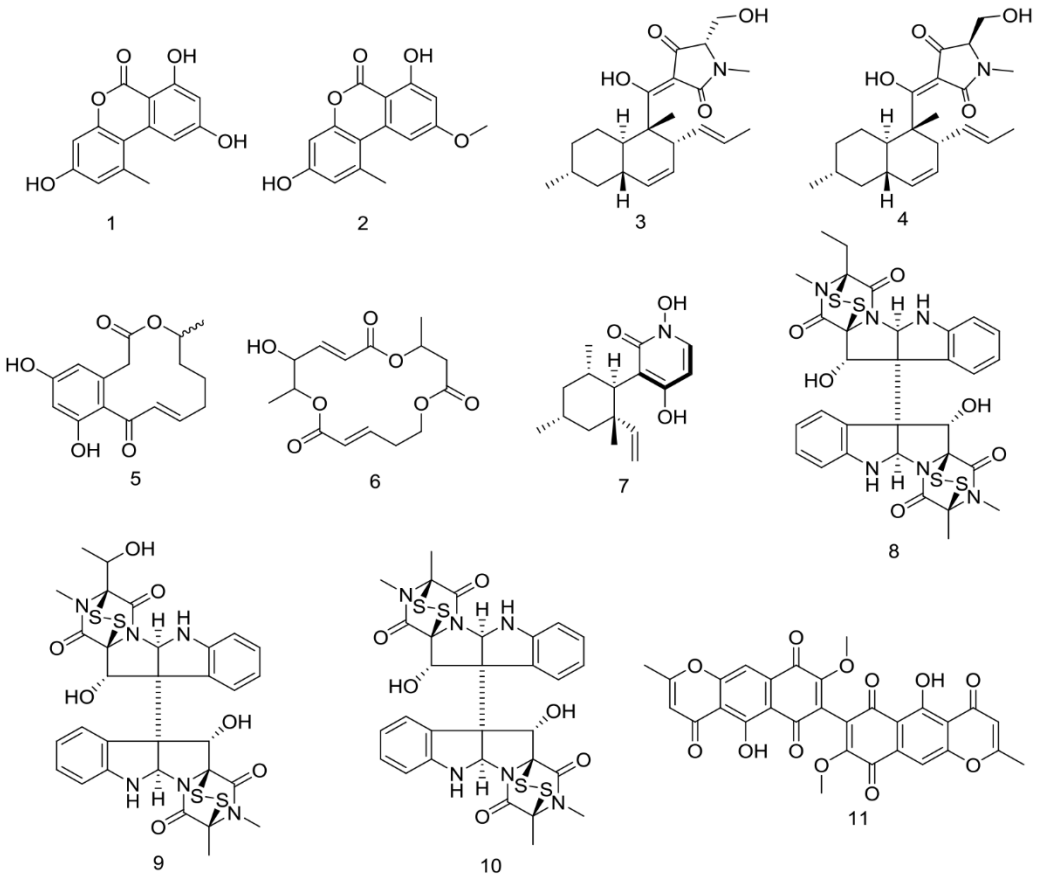
*Fungal endophyte OTUs were tentatively assigned to either genus or species by matching the most homologous sequences in GenBank by BLAST search. Mainly

authentic sequences were used for assigning OTUs preferentially from type or other authentic, and annotated cultures generated by taxonomic specialist published in high impact factor Mycology journals. When multiple species were found to have high sequence similarity, or when <97% sequence homology was found with a published authentic sequence for which a culture was deposited in a public culture collection, we choose to take a more conservative approach and use only the genus, family, order, class name for OTU assignment (see Raja et al., 2015)

Fungal Metabolites

A total of eleven secondary metabolites, all of which are known compounds, were identified from the fungal isolates (Figure 1, Table 4). The compounds alternariol **(1)**, alternariol monomethyl ether **(2)**, 5'epi-equisetin **(3)** equisetin **(4)**, 10-11 dehydrocurvularin **(5)**, macrosphelide A **(6)**, and cordipyridone A **(7)** , were isolated with chromatographic approaches and their spectroscopic data (accurate mass, ^{1}H NMR, and ^{13}C NMR) matched literature reports (Table 4). The remaining compounds, Sch 52901 **(8)**, Sch 52900 **(9)**, Verticillin A **(10)**, Aurofusarin **(11)** were identified by comparison of LC-MS data with a library of previously isolated fungal metabolites (El-Elimat et al., 2013a; Figueroa et al., 2014).

Figure 1. Metabolites from Endophytic Fungi of *Hydrastis canadensis*.

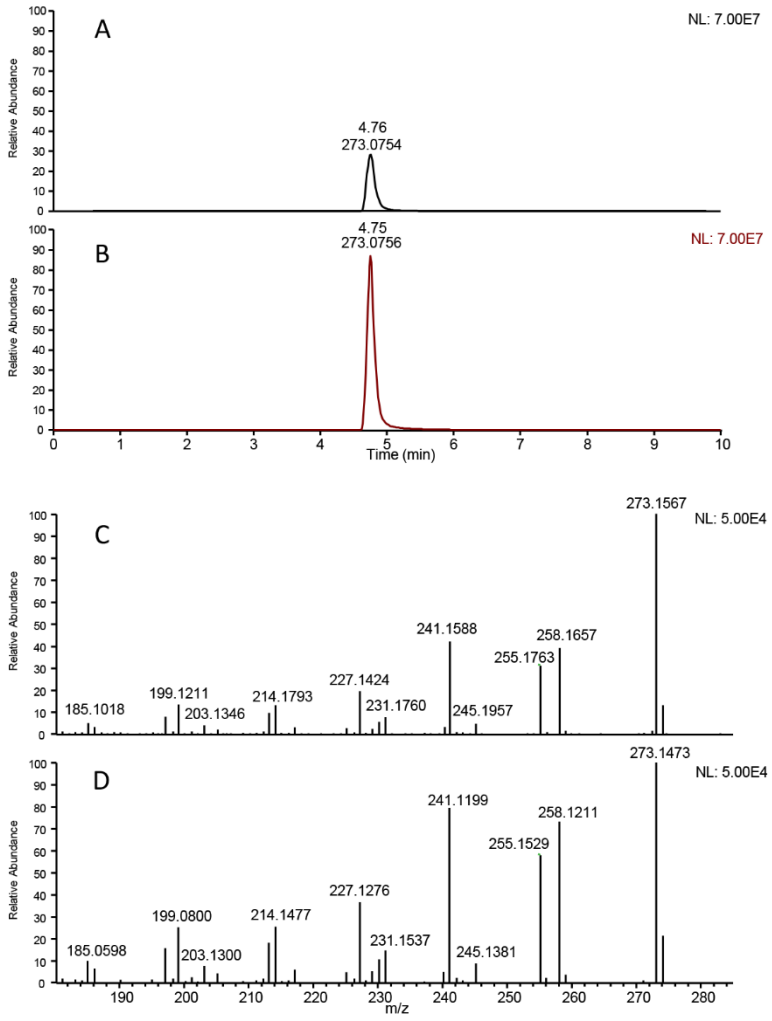


Isolated metabolites, Alternariol (1), Alternariol monomethyl ether (2), 5'-epiquisetin (3), Equisetin (4), 10-11 Didehydrocurvularin (5), Macrosphelide A (6), Cordypyridone A (7), were found to be >95% Pure by UV/Vis. Metabolites Sch 52901 (8), Sch 52900 (9), Verticillin A (10), Aurofusarin (11) were verified via UPLC-MS/MS dereplication against an in-house database.

Presence of Fungal Metabolites in Plant Material

Of the eleven metabolites identified from the fungal endophytes, one compound, alternariol monomethyl ether (**2**), was detected in a botanical extract with the methods employed here. Retention time, accurate mass ($[M+H]^+$, 273.0754, calcd for $C_{15}H_{13}O_5^+$, 273.0763), and MS-MS fragmentation pattern for the putative alternariol monomethyl ether ion in the extract matched that of isolated alternariol monomethyl ether (Figure 2). The alternariol monomethyl ether was detected in two adjacent normal phase chromatography fractions of a seed extract from *H. canadensis*. This compound was absent from other fractions of the same extract and from blank injections conducted before each extract analysis. Additionally, extracts from the other plant parts of *H. canadensis* did not contain detectable levels of alternariol monomethyl ether. Finally, alternariol monomethyl ether was also absent from another collection of *H. canadensis* seeds, which were harvested from the same plot but at a later date.

Figure 2. Selected Ion Chromatogram for m/z 273.0755 in a Concentrated Fraction From an Extract of *H. canadensis* Seeds (Analyzed at 1mg/mL)



(A) and isolated alternariol monomethyl ether from Alternaria sp. at a concentration of 1mg/mL (B). MS-MS fragmentation patterns for the precursor ion at m/z 273.0754 are in excellent agreement between the compound detected in the plant material (C) and isolated alternariol monomethyl ether (D).

Consistent with the presence of alternariol monomethyl ether in the botanical seed extract, this compound was also isolated from two *H. canadensis* seed endophytes (*Alternaria* sp., G13 and G31, Table 1). Interestingly, *Alternaria* spp. are known to exist both as endophytes and plant pathogens (Woudenberg et al., 2013). Thus, it is possible that this fungus inhabits *H. canadensis* seeds as a means of transmission to *H. canadensis* seedlings. At the same time, the fungus may confer a protective effect on the seeds by producing antimicrobial compounds. Consistent with these observations, *Alternaria* spp. were identified in collections of goldenseal from the same plot over a multiyear time course. A collection done on 6/28/2012 provided an additional case of *Alternaria* sp (G275) being identified from the seed material. ITS sequencing placed this strain of *Alternaria* sp (G275) as being highly similar to the *Alternaria* sp (G31) identified in the initial collection. Furthermore, a third collection of goldenseal from this plot done on 6/11/2015 yielded an endophyte from the stem segments that was a producer of alternariol monomethyl ether. This endophyte was noted as being morphologically similar to an *Alternaria* sp. and sequencing is currently being done to confirm the identity of the species. Additional efforts are underway for the characterization and identification of fungal endophytes from the collections done on 6/28/2012 and 6/11/2016.

Antimicrobial Activity of Fungal Metabolites and Botanical Extracts

To explore the potential relevance of alternariol monomethyl ether to the biological activity of *H. canadensis*, antimicrobial activity against *S. aureus* was measured for all of the botanical extracts and for isolated alternariol monomethyl ether. *Staphylococcus aureus* was chosen as a test case for this study because it is a common Gram-positive bacterial pathogen (Kaatz and Seo, 1995). As demonstrated in Table 2, all of the botanical extracts demonstrated weak antimicrobial activity, with minimum inhibitory concentrations (MICs) of ≥ 300 µg/mL, and IC_{50} values of ≥ 3 µg/mL. Purified alternariol monomethyl ether was slightly more active as an antimicrobial than the botanical extracts, with an MIC of 75 µg/mL.

Table 2. MIC of Extracts

Sample	MIC
Areial extract	200 µg/mL
Seed extract	> 200 µg/mL
berberine	75 µg/mL
alternariol monomethyl ether	75 µg/mL

a. MIC is defined as the concentration that caused OD600 to be reduced by ≥ 95%.
b. Concentration is expressed as µg/mL of plant extract per mL of assay well volume.

The results for quantitative analysis of alternariol monomethyl ether in the botanical extracts provide some context for the findings in Table 2. This compound was found only in a purified fraction of the *H. canadensis* seed extract, and based on the concentration in this extract, the back-calculated concentration of alternariol monomethyl ether in the original extract was found to be 991 ppm (expressed as mg of alternariol monomethyl ether per Kg of plant material). At the highest concentration of the seed extract tested in the bioassay (200 µg/mL, expressed as mg extract per L assay volume), this equates to an assay concentration of only 0.1982 µg/mL alternariol monomethyl ether, considerably below the observed MIC of 75 µg/mL for this compound. Thus, it is little surprise that the seed extract did not display marked antimicrobial activity (Table 3). However, it is worth noting that many of the fungal endophytes isolated as part of this study possessed antimicrobial activity (Table 3), presumably due to the presence of other antimicrobial metabolites. It is entirely possible that the combined effect of multiple low-

level antimicrobial fungal metabolites could contribute to the overall activity of a botanical extract. Further studies would be necessary to evaluate this possibility.

The detection of alternariol monomethyl ether in *H. canadensis* seeds suggests that the presence of the fungus may provide some benefit to the plant. Quantitative analysis of the seed extract indicated that alternariol monomethyl ether was present at a concentration of approximately 0.072 µg/mL in the extract of the seed material (36% of 0.2 µg/mL extract), which equates to 991 ppm in the *H. canadensis* seeds. This concentration is above that reported for metabolites known to be ecologically important, such as the ergot and loline alkaloids produced by fungal endophytes of native grasses. Ergot and loline alkaloid alkaloids help protect grasses from insect herbivores (Bush et al., 1997). In a similar fashion, perhaps the presence of antimicrobial fungal metabolites in *H. canadensis* seeds helps protect from plant bacterial pathogens. Exploration of this hypothesis would be another interesting avenue of further study.

CHAPTER III

METHODS

Acquisition of Plant Material

Plant material was harvested from a cultivated plot in Hendersonville, NC (N35°24.2770, W 082°20.9930) on 07/11/2011 and a voucher was deposited in the University of North Carolina Herbarium (accession number NCU 583414). Additional collections were made on 6/28/2012 and 6/11/2015 from the same plot for further analysis. For extraction, directly from the plant, the material was allowed to air dry until crisp. Leaves, roots, stems and seeds were separated and ground to a fine powder using a Thomas Wiley Mini-Mill.

Fungal Cultivation and Strain Identification

Fresh plant samples were surface sterilized as described previously (Raja et al., 2015). A total of 320 segments were plated, which included 100 stem segments; 100 leaves; 70 root segments; and 50 seeds. All fungal endophyte cultures that emerged from goldenseal plant parts are maintained on Potato Dextrose Agar; Difco (PDA) agar slants at 9˚C at the University of North Carolina at Greensboro, Department of Chemistry and Biochemistry Fungal Culture Collection. A total of 23 fungi were cultured from tissues of *H. canadensis* (collected on July 11 2011) and were grown on a rice solid-state

fermentation medium, as previously described (Raja et al., 2015). All rice cultures were allowed to grow for 14-21 days prior to chemical extraction.

For molecular identification of fungal endophytes isolated from goldenseal, the internal transcribed spacer region of the ribosomal RNA gene (ITS) was sequenced using protocols outlined previously (Raja et al. 2015) and OTU designations as surrogates for species identifications were made with BLAST search tool utilizing only authenticated, published sequences in NCBI GenBank using 98% identity (Raja et al., 2015; Schoch et al., 2014). In addition, phylogenetic analysis of the ITS region using Maximum Likelihood was employed to confirm the ITS phylogeny of isolated strains (Data not shown). The ITS sequences from all strains were deposited in GenBank and are listed in Table 1.

Extraction and Preparation

Fungal material and botanical extracts were extracted in 1:1 methanol:chloroform and subjected to liquid-liquid partitioning using previously described methods (El-Elimat et al., 2015; Kaur et al., 2016; Kellogg et al., 2016; Raja et al., 2015), resulting in a chloroform, aqueous, and hexane partitions. The chloroform partitions were of primary interest and used for further investigations.

Identification of Fungal Metabolites

Fungal extracts were analyzed using ultra performance liquid chromatography (Acquity UPLC, Waters) coupled to high resolving power tandem mass spectrometry (LTQ Orbitrap XL, Thermo) (LC-MS-MS) with previously published methods (El-Elimat et al., 2013a). The retention time, accurate mass, and fragmentation spectrum for each ion detected were compared to a library of LC-MS data for 262 fungal metabolites to identify known compounds (El-Elimat et al., 2013a). Compounds that were not represented in the existing fungal library were isolated and characterized via several rounds of flash chromatography on a Combiflash Rf system (Teledyne-ISCO, Lincoln, NE, USA) and high performance liquid chromatography on the Varian HPLC system (Agilent Technologies, Santa Clara, CA, USA), and structures for pure compounds were solved based on NMR data (Joel ECS 400Mhz, Joel ECA 500Hhz, Varian 700 MHz) and accurate mass measurements, as described previously (Raja et al., 2015). For each isolated compound, NMR and accurate mass data matched literature reports (Table 4).

The same method used to collect LC-MS-MS data of fungal extracts was also applied to botanical extracts from *H. canadensis.* To determine if fungal metabolites were detectable in the botanical extracts, the resulting LC-MS data were filtered for the *m/z* value of the $[M+H]^+$ ion of each fungal metabolite. A 5 ppm isolation window was used. For identification, comparisons were made between retention time, accurate mass, and fragmentation pattern of any ion present in both the botanical and fungal extracts. Care was taken to ensure that any fungal metabolites identified in the extracts were not a

result of accidental contamination from other fungal samples. Multiple blank injections were conducted between the analysis of each sample, and these blank injections were scrutinized to rule out carry-over.

Quantitative Analysis of Alternariol monomethyl ether

A stock solution of alternariol monomethyl ether isolated from *Alternaria* sp. (Table 4) was prepared in 50:50 methanol dioxane at a concentration of 1 µg/mL. The identity of this compound was verified by NMR, and its purity was determined to be >95% based on UPLC (Kellogg et al., 2016). Calibration solutions were prepared from the stock solution via serial dilution over a concentration range of 0.2 mg/mL to 2 ng/mL. These solutions were analyzed using the same LC-MS-MS method applied to the extracts, as described previously (El-Elimat et al., 2013a) . A calibration curve was calculated as peak area for the selected $[M+H]^+$ ion for alternariol monomethyl ether (*m/z* 273.0755) versus concentration, and alternariol monomethyl ether concentration was determined in the fungal and botanical extracts based on the best-fit line for this calibration curve.

Evaluation of Antimicrobial Activity

Clinical Laboratory Standards Institute (Ferraro, 2000) methods for broth microdilution assays were employed to evaluate the antimicrobial activity of all fungal and botanical extracts, as well as the isolated alternariol monomethyl ether. Activity was evaluated against *Staphylococcus aureus* strain SA1199 (Kaatz and Seo, 1995). The known antimicrobial compound berberine served as a positive control for these experiments(Junio et al., 2011). All samples were tested in triplicate with a final DMSO content of 2% in each well. Growth was measured based on absorbance measurements at 600 nm (OD_{600}). Minimum inhibitory concentration (MIC) was defined as concentration at which the mean OD_{600} of the test wells was reduced by ≥95%.

CHAPTER IV

CONCLUSION

The investigation into the presence of fungal components in a botanical medicine has shown that although the fungal metabolites can be detected in the original plant material. However, the concentration that they are present in is lower than what would be expected to directly contribute to the antimicrobial activity of the plant. Nonetheless, the concentration of alternariol monomethylether is higher than concentrations proven to be ecologically important for other fungal endophyte secondary metabolites, such as that of loline alkaloids, present in some grazing grasses. The findings of this study suggest that the fungal secondary metabolites found in these samples may offer some selective advantage to the botanical. The findings that *Alternaria* sp. is found in multiple collections of *H. canadensis* over a multi-year time course supports this hypothesis.

Additionally, of the 23 fungal cultures investigated, many proved to be antimicrobial against *S. aureus*, even if the compounds responsible could not be isolated. Of the 11 compounds isolated and characterized, 5 had published antimicrobial activity, and all had at least some published bioactivity. Therefore, investigations into fungal endophytes still show great promise for uncovering bioactive compounds.

Beyond this study, it is probable that other components that were not identified previously could also overlap between the fungal endophytes and the botanicals that they

inhabit. Further investigations could shed additional insights into the importance of fungal secondary metabolites for the host plants they inhabit.

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APPENDIX A

SUPPLEMENTARY DATA

Table 3. Bioactivity of Fungal Extracts against *S.aureus* at 20 µg/mL and 200 µg/mL (expressed as mass of extract per volume of assay well).

G#	OTU identification	20µg/mL[a]	200µg/mL
G09	*Diaporthe eres*	62 (± 13)	99 (± 0.6)
G10	*Diaporthe* sp.	N.I.	N.I.
G11	Sordariomycete sp. (Xylariales)	N.I.	N.I.
G12	*Colletotrichum fioriniae*	N.I.	N.I.
G13	*Alternaria* sp.	98 (± 1.7)	100 (± 3.0)
G14	*Diaporthe eres*	N.I.	94 (± 4.0)
G15	*Diaporthe eres*	N.I.	99 (±0.5)
G16	*Diaporthe* sp.	57 (± 33)	99 (± 1.2)
G17	*Diaporthe* sp.	60 (± 5.3)	98 (±1.1)
G22	*Phoma* sp.	56 (± 5.6)	99 (± 0.4)
G23	*Magnaporthales* sp.	33 (± 12)	98 (± 0.3)
G28	*Alternaria* sp.	100 (± 1.2)	100 (± 2.0)
G29	*Diaporthe* sp.	67 (± 5.5)	90 (± 0.9)
G30	*Colletotrichum fioriniae*	58 (± 19)	74 (± 25)
G31	*Alternaria* sp.	100 (± 1.1)	100 (± 3.4)
G33	*Alternaria* sp.	3.8 (± 29)	100 (± 2.3)
G34	*Colletotrichum fioriniae*	N.I.	N.I.
G35	*Colletotrichum fioriniae*	38 (± 15)	60.8 (± 40)
G36	*Alternaria* sp.	100 (± 0.4)	100 (± 2.7)
G38	*Colletotrichum fioriniae*	N.I.	100 (± 2.1)
G39	*Colletotrichum fioriniae*	16 (± 1.1)	100 (± 1.0)
G41	*Pyrenochaeta* sp.	73 (± 4.6)	73 (± 10)
G42	Sordariomycete sp. (Xylariales)	36 (± 26)	35 (± 19)

a. The known antimicrobial alkaloid berberine served as positive control for these experiments and demonstrated an MIC of 75-150ppm, consistent with literature(Amin et al., 1969; Čerňáková and Košťálová, 2002).

Table 4. Compounds from H. canadensis Endophytic Fungi with Reported Biological Activity Indicated.

Compound Name	Reported Activity	Found In[a]	Isolation[a]	Bioactivity
Alternariol (1)	Antimicrobial	G15	(Raistrick et al., 1953)	(Raistrick et al., 1953)
Alternariol monomethyl ether (2)	Antimicrobial/ Mycotoxin	G13,G31	(Bensassi et al., 2011)	(Lou et al., 2013)
5'-epiequisetin (3)	Phytotoxin	G52	(Wheeler et al., 1999)	(Wheeler et al., 1999)
Equisetin (4)	Gram + Antimicrobial	G12,G52	((Burmeister et al., 1974)	(Burmeister et al., 1974; Wheeler et al., 1999)
10,11-Dehydrocurvularin (5)	HSF1 inhibitor	G36	(Hyeon et al., 1976)	(Santagata et al., 2011)
Macrosphelide A (6)	Antibiotic	G41	(Takamatsu et al., 1996)	(Takamatsu et al., 1996)
Cordypyridone A (7)	Erythropoietin Inducer	G16	(Cai et al., 1999)	(Cai et al., 1999)
Sch 52901 (8)	Cytotoxic	G33	(Chu et al., 1995)	(Chu et al., 1995)
Sch 52900 (9)	Cytotoxic	G35	(Chu et al., 1995)	(Chu et al., 1995)
Verticillin A (10)	Cytotoxic/ Gram + Antimicrobial	G35	(Katagiri et al., 1970)	(Katagiri et al., 1970)
Aurofusarin (11)	Mycotoxin	G12,G30, G34,G38	(Dvorska et al., 2002)	(Dvorska et al., 2002)

a. Compounds 1-7 were identified by isolation and structure elucidation, while compounds 8-11 were identified by comparison with a library of LC-MS data (El-Elimat et al., 2013b).

Figure 3. Antimicrobial Activity of a Goldenseal Leaf Extract against *Staphylococcus aureus*.

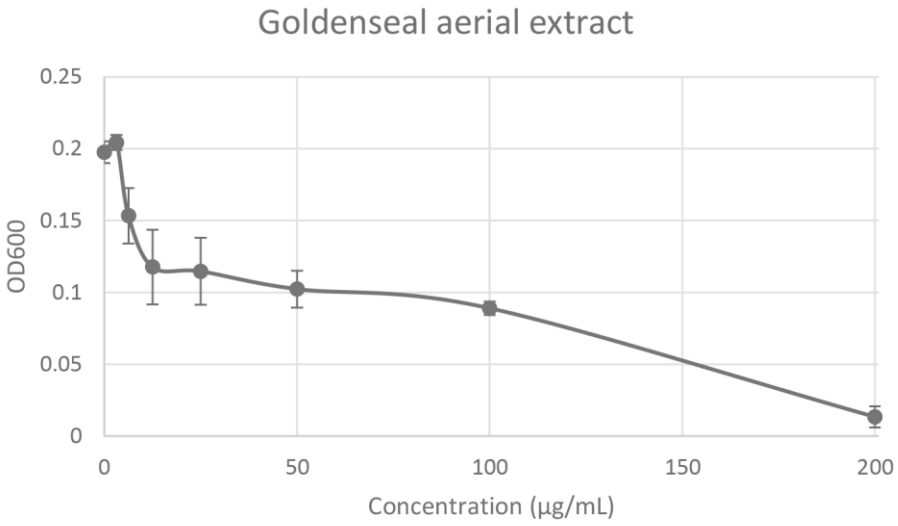

Figure 4. Antimicrobial Activity of Goldenseal Seed Extract against *Staphylococcus aureus.*

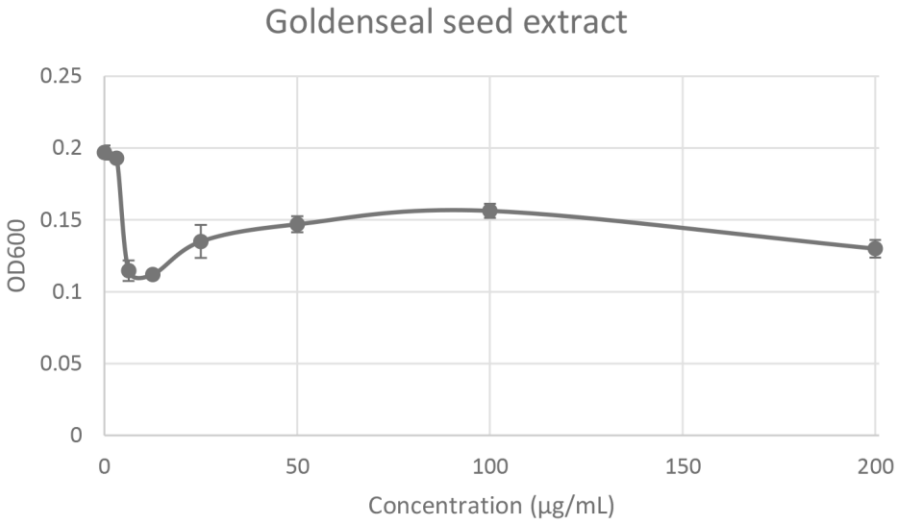

Figure 5. Antimicrobial Activity of Alternariol Monomethylether against *Staphylococcus aureus*.